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Short Communication

Anomalous Expression of P-Cadherin in Breast Carcinoma

Correlation with E-Cadherin Expression and Pathological Features

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Previous studies on the cell-cell adhesion molecules P- and E-cadherin have shown that P-cadherin is not expressed in breast cancer. In contrast, the expression of E-cadherin is a normal event in these tumors, but a reduction in the levels of this molecule in neoplastic cells is associated with the histological type, high histological grade, greater tumor size, and metastasis. The expression pattern of P- and E-cadherin were immunohistochemically studied in tissue sections from normal breast tissue, benign breast lesions, and 57 infiltrating breast carcinomas. Cadherin expression was analyzed in parallel with pathological features and the immunohistochemical expression of estrogen and progesterone receptors in breast carcinomas. P-cadherin was detected in the myoepithelial cells and E-cadherin in luminal epithelial cells from normal breast and benign breast lesions. P-cadherin expression was detected in 9 of 45 cases (20%) of infiltrating ductal carcinomas of no special type; none of the special histological types that were analyzed (7 infiltrating lobular carcinomas, 3 colloid carcinomas, and 2 infiltrating papillary carcinomas) expressed P-cadherin. In infiltrating ductal carcinomas, P-cadherin expression correlated significantly with a reduction in E-cadherin expression, histological grade (all cases were grade III tumors), and hormone receptor content (8 of 9 cases were estrogen and progesterone receptor negative). Although E-cadherin was not found in the 7 infiltrating lobular carcinomas, it was present in the remaining histological types and was preserved in 15 infiltrating ductal and 3 colloid and 2 papillary carcinomas and was reduced in 30 infiltrating ductal carcinomas. In addition, a reduction in E-cadherin expression was significantly associated with high histological grade and a lack of steroid hormone receptors in infiltrating ductal carcinomas. No apparent relationship was found between P- and E-cadherin expression and tumor size and axillary lymph node metastasis. The distinct patterns of P- and E-cadherin expression observed in this study strongly suggest a differential role for these cadherins in human breast carcinogenesis. (Am J Pathol 1995, 146:605–612)

The maintenance of adult tissue architecture largely depends on the structural and functional integrity of cadherins, a superfamily of Ca^{2+}-dependent cell-cell adhesion molecules that usually mediate homophilic

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and homotypic intercellular adhesion.[1,2] Classical E-(epithelial), and P-(placental) cadherins (CDs), which are preferentially located at the adherens type of intercellular junctions,[3] share a common basic structure but have different molecular masses, binding specificities, and tissue distribution.[4] Immunohistochemical studies have demonstrated that human E-CD is expressed in most epithelial tissues, whereas P-CD is restricted to the basal or lower layers of stratified epithelia, where it is frequently coexpressed with E-CD.[5] In breast tissue, E-CD is expressed in epithelial luminal cells, whereas P-CD is expressed in myoepithelial cells.[5,6]

The role of E-CD in carcinogenesis has been studied extensively in the last few years. Observations in experimental and human carcinomas have suggested that reduced E-CD expression induces dedifferentiation, tumorigenicity, and invasiveness in carcinoma cells.[7–9] In breast carcinomas, a relationship has been observed between a reduction in E-CD expression and the histological type and grade, tumor size, and metastasis.[10–12] In contrast, as few studies on P-CD have been done until now,[5,6,13–15] the expression and role of this cadherin in human carcinomas is not known in detail.

To analyze the possible role of P-CD in breast carcinogenesis, we studied the expression of this cell-cell adhesion molecule in a series of breast carcinomas. In addition, we investigated the relationship between the expression of P-CD and E-CD, histological grade, tumor size, axillary lymph node metastasis, and hormone receptors content. These two molecules are differentially expressed in infiltrating ductal carcinomas, as P-CD expression is detected only in tumors with reduced E-CD expression. In addition, P-CD expression is significantly associated with a high histological grade and a lack of estrogen and progesterone receptors in a subset of breast carcinomas with a characteristic growth pattern.

Materials and Methods

Specimens

Breast tissue was obtained from 57 unselected mastectomy specimens resected for infiltrating breast carcinomas and from 5 breast biopsies corresponding to 3 fibroadenomas, a complex sclerosing lesion, and a sclerosing adenosis sent to the Department of Pathology, La Paz Hospital, Madrid. None of these cases were included in a previous study on E-CD expression.[10] Neoplastic and non-neoplastic breast tissue samples were embedded in OCT compound (Miles Laboratory, Naperville, IL), snap-frozen in liquid nitrogen-cooled isopentane, and stored at −70 C. The remaining breast tissue and axillary lymph nodes were routinely fixed in 10% formalin for 24 hours and embedded in paraffin.

Antibodies

NCC-CAD-299 is a mouse monoclonal antibody that recognizes human P-CD.[5] ECCD-2 is a rat monoclonal antibody against mouse E-CD, which also recognizes E-CD in the human mammary tumor cell line MCF-7[16] and in human breast carcinomas.[10] NCC-CAD-299 and ECCD-2 were a generous gift of M. Takeichi, Kyoto University, Japan.

The mouse monoclonal antibodies CAM 5.2 (Becton Dickinson, San Jose, CA) 34βE12 (Enzo Diagnostic, New York, NY), and K068 (Biømeda, Foster City, CA) were used to study the expression of low molecular weight cytokeratins (8, 18, and 19), high molecular weight cytokeratins (1, 5, 10, and 14) and cytokeratin 7, respectively. The mouse monoclonal antibody HHF35 (Enzo Diagnostic) recognizes muscular actin. S-100 expression was studied with the rabbit anti-cow S-100 polyclonal antibody (Dako A/S, Glostrup, Denmark)

The immunohistochemical localization of nuclear estrogen and progesterone receptors was performed with the ER-ICA and PgR-ICA kits (Abbott Laboratories, North Chicago, IL).

Immunohistochemical Staining

Immunostaining for P- and E-CD was performed by the avidin-biotin-alkaline phosphatase method, as previously reported,[10,17] with some minor modifications. Briefly, cryostat sections of 5 to 6 μ thickness were cut, fixed in 10% formalin in Tris buffer containing 10 mmol/L Ca^{2+}, pH 7.2, for 3 minutes and post-fixed at −20 C in methanol for 1 minute and in acetone for 3 minutes. After washing, nonspecific antibody binding was blocked with 5% (w/v) nonfat milk, 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO). The primary antibodies NCC-CAD-299 and ECCD-2 were applied at a dilution of 1:20 and 1:200, respectively. Primary antibody dilutions were carried out in 150 mmol/L NaCl, 10 mmol/L HEPES, pH 7.4, 10 mmol/L $CaCl_2$ (HMF-Ca buffer), containing 1% bovine serum albumin (Sigma). After washing in Tris buffer, tissue sections were incubated with biotinylated rabbit anti-mouse and rabbit anti-rat immunoglobulins (Dako A/S) and then incubated with streptavidin-alkaline

phosphatase complex (Dako A/S). The alkaline phosphatase activity was developed with naphtol AS-MX phosphate as substrate and fast red as the chromogen group (Sigma). The sections were finally counterstained with Mayer hematoxylin.

Normal human skin was used as a positive control. These samples showed intense P-CD immunoreactivity along the cell-cell contacts of basal keratinocytes, whereas E-CD immunostaining was localized on the lateral and upper surfaces of basal keratinocytes and all around the periphery of keratinocytes in the spinous layer. In negative controls the primary antibody was omitted.

Immunostaining for cytokeratins, S-100, and muscle actin was performed on formalin-fixed paraffin-embedded tissue sections by the avidin-biotin-alkaline phosphatase method. The ER-ICA and PgR-ICA kits were used on frozen sections according to the manufacturer's indications, but the sections were finally counterstained with ethyl green (Cell Analysis Systems, Elmhurst, IL).

Evaluation of Immunohistochemical Staining

Positive cadherin expression was considered only when linear membrane staining was observed. In breast carcinomas, a semiquantitative estimation of P- and E-CD expression was performed by using a composite score obtained by adding the values of the immunoreaction intensity and relative abundance of cadherin immunoreactive cells, as previously reported for E-CD.[10]

Estrogen and progesterone receptor contents were evaluated by using the quantitative estrogen progesterone application (version 2.0) for the CAS 200 analyzer (Cell Analysis Systems, Lombard, IL). Sampling for measurements was performed according to Esteban et al.[18] A tumor was considered positive for estrogen and/or progesterone receptors when the positive nuclear area was $\geq$10%.

Carcinoma histological typing was performed on formalin-fixed and paraffin-embedded samples. The combined histological grade (I, II, and III) of infiltrating ductal carcinomas was obtained according to Elston.[19] Tumor size and lymph node status (0 versus positive axillary lymph nodes) were also evaluated.

The χ^2 test was used to analyze the statistical significance of the relationship between P- and E-CD expression and tumor size, lymph node metastasis, and hormone receptor content.

Results

Cadherin Expression in Normal Breast Tissue and Benign Breast Lesions

In normal breast tissue P-CD was strongly expressed in the membrane surface of myoepithelial cells (Figure 1A). Lobular and ductal epithelium expressed E-CD in a regular array on lateral cell borders. In benign lesions, the immunoreactivity pattern for both cadherins was similar to those observed in normal breast tissue (Figure 1B).

Cadherin Expression and Breast Carcinoma Histological Type

Of the 57 samples analyzed, 45 cases were infiltrating ductal carcinomas of no special type (IDCNST), 7 were infiltrating lobular carcinomas (ILC), 3 were colloid carcinomas, and 2 were infiltrating papillary carcinomas. Of the 45 IDCNST, 9 (20%) showed P-CD membrane immunostaining (Figure 1D, E). Intense or moderate immunoreactivity was observed in all 9 cases, and the percentage of immunoreactive cells ranged from 10 to 90%. Thus, the 9 cases showed a composite score of 4 to 7 (Table 1). None of the 12 carcinomas of special histological type expressed P-CD (Figure 1F). In most of the 57 breast carcinoma samples analyzed, staining for P-CD was observed in myoepithelial cells around areas of carcinoma *in situ* (Figure 1F) or in normal breast parenchyma adjacent to the carcinomas.

E-CD immunoreactivity was observed in all but 5 IDCNST. The range of E-CD immunoreactivity in the remaining tumors was very wide; 5 cases had scores of 2 to 3, 19 cases had scores of 4 to 5, and the remaining 15 cases had scores of 6 to 7. The 7 ILC showed no E-CD immunoreactivity whereas the 3 colloid and 2 papillary carcinomas showed E-CD expression with scores of 6 or 7.

For statistical analysis, IDCNSTs were subdivided into cases with present or absent P-CD expression and cases with preserved or reduced E-CD expression. Present P-CD expression indicates any degree of P-CD immunoreactivity (9 of 45 IDCNST). Cases with an E-CD composite score of 6 and 7 were considered to have preserved E-CD expression (15 cases of 45 IDCNST). In contrast, cases with scores of 0 to 5 were considered to have reduced E-CD expression (30 cases of 45 IDCNST). The expression of both cadherins was classified in different ways because E-CD is expressed in normal breast epithelium and a reduction in the levels of E-CD expression in

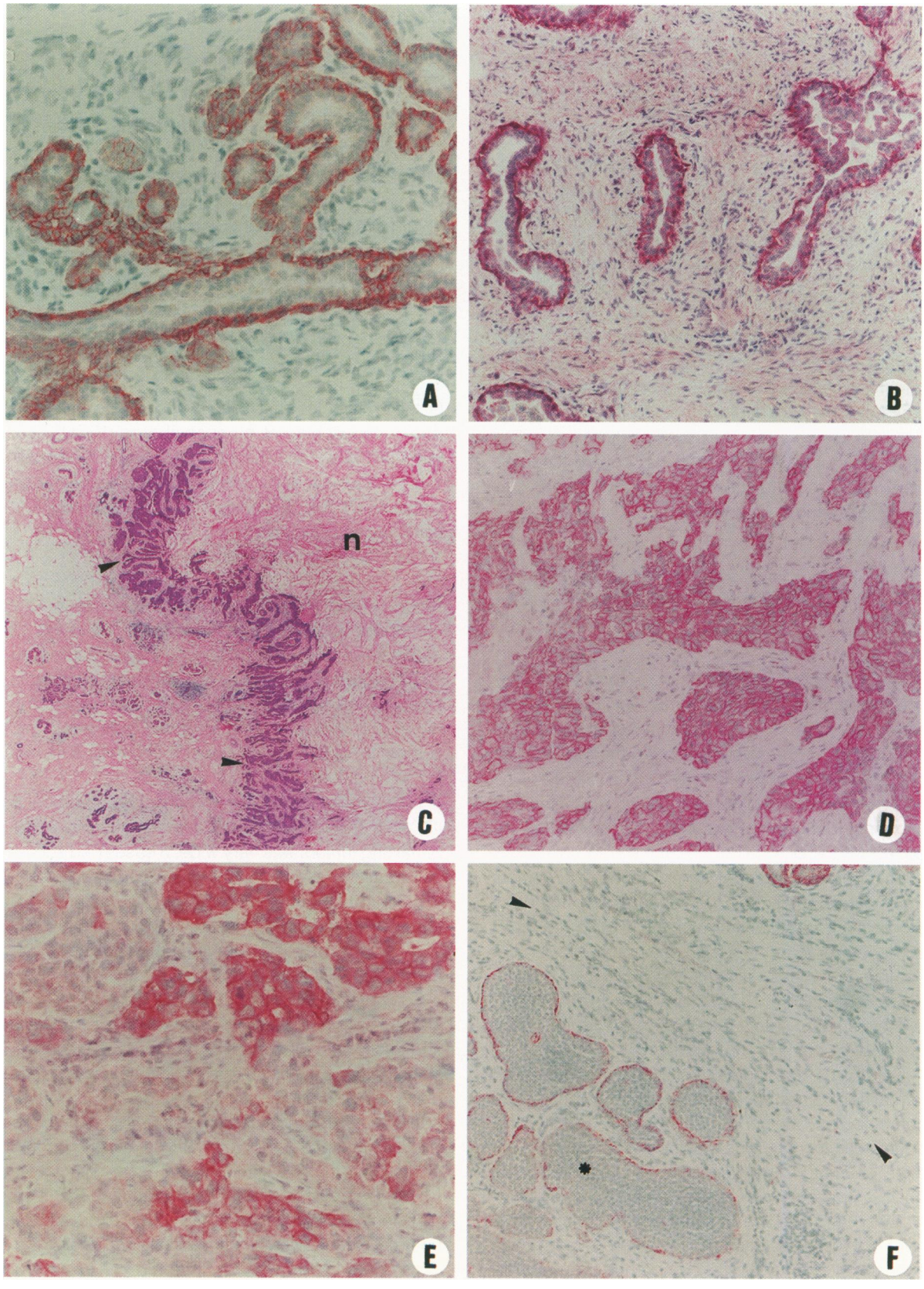
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Table 1. *Clinicopathological Features in IDCNST that Expressed P-CD*

Patient	Age (years)	Tumor size (mm)	P-CD expression*	E-CD expression*	Axillary lymph node metastasis
1	64	17	4 (3+1)	4 (2+2)	0
2	64	8	5 (2+3)	0	0
3	52	30	7 (3+4)	5 (3+2)	5
4	56	17	6 (3+3)	4 (2+2)	0
5	49	31	6 (3+3)	2 (1+1)	0
6	40	15	4 (2+2)	4 (2+2)	2
7	44	30	5 (2+3)	4 (2+2)	
8	50	32	5 (2+3)	2 (1+1)	14
9	36	20	5 (3+2)	5 (3+2)	0

All cases were grade III carcinomas. All carcinomas but tumor in patient 2 were negative for estrogen and progesterone receptor.
*Composite score obtained by adding the intensity of immunoreaction (0 to 3) and relative abundance of positive cells (0 to 4).

breast carcinoma cells has been thought to play a role in invasion and metastasis.[11] In contrast, P-CD expression is seen only in the myoepithelial cells of normal breast, and any degree of P-CD expression must be considered anomalous in ductal breast carcinoma.

The relationship between P- and E-CD expression in IDCNST is shown in Table 2. E-CD expression was reduced in all of the 9 tumors that expressed P-CD but in 21 of the 36 tumors without P-CD expression ($P < 0.01$).

Table 2. *Correlation between P- and E-CD Expression in IDCNST of the Breast*

	P-CD present	P-CD absent
E-CD preserved	0	15 (100%)
E-CD reduced	9 (30%)	21 (70%)

χ^2 test; $P < 0.01$.

P-CD Expression and Tumor Growth Pattern and Cytokeratin, S-100, and Muscle Actin Expression in IDCNST

In seven of the nine cases that expressed P-CD, the tumor had a central area of necrosis and/or fibrosclerosis. Tumor cells grew only at the periphery of the lesion where they formed infiltrative nests and trabeculae (Figure 1C). Only one of the remaining cases had occasional patches of tubular formation. In none of the cases were there histological signs of myoepithelial differentiation, such as tubular structures with a double cellular component or clear and/or fusiform cells. To further analyze a possible myoepithelial differentiation in these P-CD-positive breast carcinomas, we studied the expression pattern of cytokeratins, S-100, and muscle actin. All cases reacted extensively with low molecular weight cytokeratins and cytokeratin 7 antibodies, but no immunoreaction was observed with the high molecular weight cytokeratins monoclonal antibody. Cytoplasmic and nuclear S-100 immunostaining was observed in between 10 and 75% of the cells in five cases. Positivity to muscle actin was restricted to less than 5% of the cells in only two cases.

Cadherin Expression and Pathological Features and Hormone Receptor Content in IDCNST

Table 3 shows the relationship between P- and E-CD expression and histological grade, tumor size, lymph node metastasis, and hormone receptor content. A significant correlation was found between cadherin expression and tumor grade, as all cases with P-CD expression were grade III carcinomas, and the frequency of reduced E-CD expression was higher in these cases than in the grade I and II tumors. No significant correlation was observed between cadherin expression and tumor size or axillary lymph node metastasis. In IDCNST, cadherin expression significantly correlated with the content of estrogen and progesterone receptors. Cases without hormone receptors were more frequent in breast carcinomas that expressed P-CD or showed reduced E-CD expression.

Figure 1. *Patterns of P-CD expression in normal breast tissue, benign breast lesions, and infiltrating breast carcinomas.* **A:** *Membrane P-CD immunoreactivity in myoepithelial cells of normal breast ducts and acini.* **B:** *P-CD immunostaining is also restricted to myoepithelial cells in this example of fibroadenoma.* **C** *Characteristic growth pattern of breast carcinomas that express P-CD. A central area of necrosis (n) is surrounded by proliferating trabeculae and tumor cell nest (arrowheads) (H&E stain).* **D:** *Intense P-CD immunostaining is seen in tumor cell nests from a grade III infiltrating ductal carcinoma.* **E:** *Intense but heterogeneous membrane immunostaining of P-CD in a poorly differentiated ductal breast carcinoma.* **F:** *Absent P-CD expression in the* in situ (*) *and infiltrating (arrowheads) components of an infiltrating lobular breast carcinoma. Strong P-CD immunoreactivity is seen in myoepithelial cells of ducts and distended acini. Original magnification, ×100 in* **A** *and* **E**, *×20 in* **B**, *×60 in* **C** *and* **F**, *and ×200 in* **D**.

Table 3. *Correlation of P- and E-CD Expression with Clinicopathological Features and Hormone Receptor Content in IDCNST of the Breast*

	P-CD expression			E-CD expression		
	Present	Absent	*P* value	Preserved	Reduced	*P* value
Tumor grade (*n* = 45)						
I	0	10 (100%)		6 (60%)	4 (40%)	
II	0	12 (100%)	<0.01	5 (42%)	7 (58%)	<0.05
III	9 (39%)	14 (61%)		4 (17%)	19 (82%)	
Tumor size (*n* = 45)						
≤20 mm	5 (17%)	24 (83%)		11 (38%)	18 (62%)	
>20 mm	4 (25%)	12 (75%)	NS	4 (25%)	12 (75%)	NS
Lymph node metastasis (*n* = 41)						
0	6 (26%)	17 (74%)		7 (30%)	16 (70%)	
≥1	2 (11%)	16 (89%)	NS	7 (39%)	11 (61%)	NS
ER-ICA*						
+	1 (4%)	25 (96%)		13 (50%)	13 (50%)	
−	8 (43%)	11 (57%)	<0.01	2 (11%)	17 (89%)	<0.01
PgR-ICA*						
+	1 (5%)	20 (95%)		11 (52%)	10 (48%)	
−	8 (33%)	16 (67%)	<0.05	4 (26%)	20 (74%)	<0.05

χ^2 test; NS, not significant.
*Immunohistochemical localization of nuclear estrogen and progesterone receptors (see *Materials and Methods*).

Discussion

As in previous reports,[5,6] the present study has shown that P- and E-CD are differentially expressed in normal breast tissue; P-CD is expressed only in myoepithelial cells and E-CD is expressed in epithelial cells. As this pattern of cadherin expression is preserved in benign lesions, such as fibroadenomas and adenosis, P-CD could be useful in the identification of myoepithelial cells in benign pseudoinfiltrative lesions.

Present results also suggest that the expression of P-CD could be related to the histological type of breast carcinoma. P-CD expression was observed in 9 of 45 IDCNST (20%), but in none of the tumors with special histological type (7 ILC, 3 colloid carcinomas, and 2 infiltrating papillary carcinomas). In addition, P-CD expression correlated significantly with the histological grade of the IDCNST, as all of the positive cases were grade III tumors. Our results contrast with the only previous study on P-CD expression in breast carcinomas by Rasbridge et al.[6] These authors, using the monoclonal antibody NCC-CAD-299 in frozen tissue, analyzed a series of 12 ILC and 13 IDCNST (4 grade I, 4 grade II, and 5 grade III tumors) and found P-CD expression only in a solid ILC. As P-CD expression was observed only in grade III IDCNST in the present series and we did not study any ILC variant, discrepancies may be due to differences in the sample size and histological types studied between the two series.

As P-CD is expressed only in myoepithelial cells in the normal breast, the presence of this molecule in breast carcinomas might indicate myoepithelial differentiation. To investigate this hypothesis, the expression patterns of cytokeratins, S-100, and muscle actin were also studied. No P-CD-positive IDCNST expressed cytokeratins 5 and 14, which are normally present in myoepithelial cells, but all of them expressed cytokeratins typical of normal inner epithelial cells of mammary ducts and acini, such as cytokeratins 7, 8, 18, and 19. Five cases showed different levels of S-100 positivity. However, the discriminatory value of S-100 expression as a marker of myoepithelial differentiation seems to be poor as S-100 positivity has been reported in approximately 45% of infiltrating ductal carcinomas.[20] Only two of our nine P-CD-positive tumors showed focal immunoreactivity for smooth muscle actin, and this was found in less than 5% of the carcinoma cells. This could either be true myoepithelial differentiation or an aberrant expression of a myoepithelial marker in neoplastic epithelial cells. These immunohistochemical data, plus the lack of histological evidence for myoepithelial differentiation, seem to indicate that P-CD expression in ductal breast carcinomas is not specifically associated with myoepithelial differentiation. However, the pattern of P-CD expression in myoepithelial breast tumors such as adenomyoepithelioma and malignant myoepithelioma should also be explored.

It has been suggested that the expression of P-CD in carcinomas derived from epithelium that normally does not express P-CD may indicate the proliferative ability of these tumors.[13] Supporting this hypothesis, the P-CD-positive breast carcinomas did have a particular growth pattern characterized by nests and trabeculae of tumor cells with a high mitotic index and grew around a central necrotic area. These tumor nests also showed reduced E-CD expression. These findings suggest that in some highly proliferative

breast carcinomas, when E-CD is down-regulated, the formation and maintenance of cancer cell nests might be mainly mediated by P-CD, the type of cadherin that is present in the highly proliferative basal layer of the stratified adult epithelia[5] and in some embryonic tissues.[21] Interestingly, *in vitro* studies have revealed that the catenin-mediated bond between cadherins and the actin-based cytoskeleton is weaker for P-CD than for E-CD,[22] suggesting that P-CD would mediate unstable cell-cell contacts that are easily broken and reformed, as occurs in the basal layer of stratified epithelia when the basal cells change their relative position in the tissue during migration into the suprabasal layer.

Another explanation for the expression of P-CD in breast carcinomas would be to consider it a member of the oncofetal protein family, as previously suggested by Shimoyama and Hirohashi.[13] These authors observed that P-CD was expressed in gastric carcinomas and in the foregut at the neurulation stage in embryos, but only weakly and focally in the adult normal gastrointestinal epithelium. Although there are no studies on P-CD expression during breast development, P-CD is expressed in the fetal skin from which the mammary anlagen develops, and plays an important role during the development of skin appendages.[23]

The reported frequency of P-CD expression and its prognostic implications varies in relation to the primary tumor site for human carcinomas. Shimoyama et al.[5] reported P-CD expression in all 44 lung cancers examined (21 adenocarcinomas, 16 squamous cell carcinomas, 4 large cell carcinomas, 4 small cell carcinomas, and 1 carcinoid tumor). In their series, P-CD expression was weaker in well differentiated adenocarcinomas than in the poorly differentiated ones. We also recently observed P-CD expression in all of 32 cases of basal cell carcinoma of the skin including both expansive and infiltrative tumors (unpublished data). However, P-CD expression was noted in approximately 50% of the cases of gastric carcinomas,[13,14] in which the expression was more frequently observed in the well differentiated tumors than in the poorly differentiated ones, and the authors suggested that reduction of P-CD expression was related to tumor progression.[14] Similarly, a complete loss of P-CD occurs in poorly differentiated gingival squamous cell carcinoma.[15] In contrast, our study demonstrated that P-CD expression is less frequent in breast carcinoma and is strongly associated with poorly differentiated IDCNST, frequently negative for any hormone receptor. Thus, although no correlation was found between P-CD expression, tumor size, and lymph node metastasis in our series, P-CD expression may identify a subset of infiltrating ductal carcinomas with a particularly poor prognosis. Additional studies are needed to investigate this suggestion in future follow-up studies with larger series.

The expression of E-CD has been analyzed in several breast carcinoma series.[6,10–12,13,24,25] Expression of E-CD is strongly associated with histological type, as most of the ILC analyzed had completely lost E-CD,[6,10–12] suggesting that the characteristic infiltrative pattern of the tumor is due to the absence of this intercellular adhesion molecule. On the other hand, most infiltrating ductal carcinomas expressed E-CD, but a decrease of E-CD expression is a frequent finding in grade III tumors.[10,11] No correlation between decreased E-CD expression and tumor size or metastasis have been found in this series or in an earlier one from our laboratory.[10] However, Oka et al[11] have reported such a relationship, suggesting that inhibition of E-CD function could enhance the possibility of release of cancer cells from the primary site in breast cancer.

Previous studies have found no correlation between E-CD expression and biochemically assessed estrogen and progesterone receptor expression in ductal breast carcinoma.[10,11] However, the present study has found that the frequency of tumors that are immunohistochemically positive for estrogen and progesterone receptors is significantly higher in cases with preserved E-CD expression than in those with reduced E-CD expression. These data suggest that steroid hormones may modulate E-CD expression, at least in part. For example, rat granulosa cells elevate their E-CD levels in response to estradiol.[26] Thus, determining whether E-CD expression is hormonally regulated in breast cancer is of interest and requires study in a larger series.

In summary, in this series P-CD was expressed only in a subset of high grade breast carcinomas that also presented reduced E-CD expression and were frequently negative for hormone receptors. The distinct patterns of P- and E-CD expression observed in this study strongly suggest a differential role for both cadherins in human breast carcinogenesis. Additional studies are needed to evaluate the possible usefulness of these cadherins as prognostic markers in breast cancer.

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